Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application.

1-38. (cancelled)

39. (previously presented) A method for selecting a nucleic acid molecule encoding a target epitope of cytotoxic T-lymphocytes, comprising:

(a) contacting host cells with cytotoxic T-lymphocytes specific for said target epitope under conditions wherein a host cell expressing said target epitope undergoes a lytic event upon contact with said cytotoxic T-lymphocytes; wherein said host cells comprise a library of heterologous nucleic acid molecules, at least one of said heterologous nucleic acid molecules encoding said target epitope, wherein said library is constructed in a vector which expresses said target epitope in said host cells, wherein said host cells express a defined MHC molecule, and wherein said cytotoxic T-lymphocytes are restricted for said MHC molecule; and

(b) recovering vector from floating host cells which are undergoing a lytic event;

wherein said target epitope is selected from the group consisting of: a target epitope which is differentially expressed in infected cells and a target epitope which is specific for an autoimmune disease.

40. (previously presented) The method of claim 39, wherein said target epitope is differentially expressed in infected cells.

41. (previously presented) The method of claim 40, wherein said infected cells are infected with a pathogen selected from the group consisting of: a virus, a fungus, and a mycobacterium.

42. (previously presented) The method of claim 41, wherein said pathogen is a virus.

43. (previously presented) The method of claim 41, wherein said infected cells are infected with a fungus.

44. (previously presented) The method of claim 41, wherein said infected cells are infected with a mycobacterium.

45. (previously presented) The method of claim 39, further comprising purifying said vector.

46. (previously presented) The method of claim 39, further comprising:

(c) purifying said vector;

(d) transferring said vector to a population of host cells, wherein said vector expresses said target epitope in said host cells, and wherein said host cells express a defined MHC molecule;

(e) contacting said host cells with cytotoxic T-lymphocytes specific for said target epitope and restricted for said MHC molecule, under conditions wherein a host cell expressing said target epitope will undergo a lytic event upon contact with said cytotoxic T-lymphocytes; and

(f) recovering vector from floating host cells which are undergoing a lytic event.

47. (previously presented) The method of claim 39, wherein said vector is a virus.

48. (previously presented) The method of claim 47, wherein said vector is a virus capable of producing infectious viral particles in eukaryotic cells.

49. (previously presented) The method of claim 48, wherein the naturally-occurring genome of said viral vector is linear, double stranded DNA.

50. (previously presented) The method of claim 48, wherein said viral vector is capable of producing infectious viral particles in mammalian cells.

51. (previously presented) The method of claim 50, wherein the naturally-occurring genome of said viral vector is linear, double-stranded DNA.

52. (previously presented) The method of claim 48, wherein said viral vector is a poxvirus vector.

53. (currently amended) The method of claim 52, wherein said poxvirus ~~viral~~ vector is a vaccinia virus ~~poxvirus~~ vector.

54. (previously presented) The method of claim 47, wherein said host cells are permissive for the production of infectious viral particles of said viral vector.

55. (previously presented) The method of claim 52, wherein said viral vector further comprises a transcriptional control signal in operable association with said heterologous nucleic acid molecules, and wherein said transcriptional control signal functions in a poxvirus.

56. (previously presented) The method of claim 55, wherein said transcriptional control signal comprises a promoter.

57. (previously presented) The method of claim 56, wherein said promoter is constitutive.

58. (previously presented) The method of claim 56, wherein said promoter is selected from the group consisting of: a vaccinia virus p7.5 promoter and a synthetic early/late promoter.

59. (previously presented) The method of claim 55, wherein said transcriptional control signal comprises a transcriptional termination signal.

60. (previously presented) The method of claim 55, wherein said vector further comprises a translational control signal associated with said transcriptional control signal.

61. (previously presented) The method of claim 60, wherein said translational control signal comprises a translation initiation codon operably linked to said heterologous nucleic acid molecules.

62. (previously presented) The method of claim 61, wherein said translation initiation codon occurs in one of three reading frames.

63. (previously presented) The method of claim 49, wherein said library is constructed by a method comprising:

(a) cleaving an isolated linear DNA virus genome to produce a first viral fragment and a second viral fragment, wherein said first fragment is nonhomologous with said second fragment;

(b) providing a population of transfer plasmids comprising said heterologous nucleic acid molecules flanked by a 5' flanking region and a 3' flanking region, wherein said 5' flanking region is homologous to said first viral fragment and said

3' flanking region is homologous to said second viral fragment; and wherein said transfer plasmids are capable of homologous recombination with said first and second viral fragments such that a viable virus genome is formed;

(c) introducing said transfer plasmids and said first and second viral fragments into a host cell under conditions wherein a transfer plasmid and said viral fragments undergo in vivo homologous recombination, thereby producing a viable modified virus genome comprising a heterologous nucleic acid molecule; and

(d) recovering said modified virus genome.

64. (previously presented) The method of claim 63, wherein said virus genome comprises a first recognition site for a first restriction endonuclease and a second recognition site for a second restriction endonuclease; and wherein said first and second viral fragments are produced by digesting said viral genome with said first restriction endonuclease and said second restriction endonuclease, and isolating said first and second viral fragments.

65. (previously presented) The method of claim 64, wherein said first and second recognition sites are physically arranged in said genome such that the region extending between said first and second viral fragments is not essential for virus infectivity.

66. (previously presented) The method of claim 63, wherein said modified virus genome is packaged in an infectious viral particle.

67. (previously presented) The method of claim 51, wherein said library is constructed by a method comprising:

(a) cleaving an isolated linear DNA virus genome to produce a first viral fragment and a second viral fragment, wherein said first fragment is nonhomologous with said second fragment;

(b) providing a population of transfer plasmids comprising said heterologous nucleic acid molecules flanked by a 5' flanking region and a 3' flanking region, wherein said 5' flanking region is homologous to said first viral fragment and said 3' flanking region is homologous to said second viral fragment; and wherein said transfer plasmids are capable of homologous recombination with said first and second viral fragments such that a viable virus genome is formed;

(c) introducing said transfer plasmids and said first and second viral fragments into a host cell under conditions wherein a transfer plasmid and said viral fragments undergo in vivo homologous recombination, thereby producing a viable modified virus genome comprising a heterologous nucleic acid molecule; and

(d) recovering said modified virus genome.

68. (previously presented) The method of claim 67, wherein said virus genome comprises a first recognition site for a first restriction endonuclease and a second recognition site for a second restriction endonuclease; and wherein said first and second viral fragments are produced by digesting said viral genome with said first restriction endonuclease and said second restriction endonuclease, and isolating said first and second viral fragments.

69. (previously presented) The method of claim 68, wherein said first and second recognition sites are physically arranged in said genome such that the region

extending between said first and second viral fragments is not essential for virus infectivity.

70. (previously presented) The method of claim 67, wherein said isolated virus genome is a poxvirus genome.

71. (previously presented) The method of claim 70, wherein said poxvirus genome is a vaccinia virus genome.

72. (previously presented) The method of claim 70, wherein said transfer plasmids and said first and second viral fragments are introduced into a host cell comprising a helper virus, wherein said host cell is non-permissive for the production of infectious virus particles of said helper virus.

73. (previously presented) The method of claim 72, wherein said helper virus is an avipoxvirus.

74. (previously presented) The method of claim 73, wherein said avipoxvirus is a fowlpox virus.

75. (previously presented) The method of claim 68, wherein said first and second restriction enzyme recognition sites are situated in a thymidine kinase gene.

76. (previously presented) The method of claim 70, wherein said first and second restriction enzyme recognition sites are situated in a vaccinia virus HindIII J fragment.

77. (previously presented) The method of claim 76, wherein said first and second restriction enzyme recognition sites are situated in a vaccinia virus thymidine kinase gene.

78. (previously presented) The method of claim 76, wherein said first restriction enzyme is NotI, and wherein said first restriction enzyme recognition site is GCGGCCGC.

79. (previously presented) The method of claim 76, wherein said second restriction enzyme site is ApaI, and wherein said second restriction enzyme recognition site is GGGCCC.

80. (previously presented) The method of claim 71, wherein said isolated virus genome is a v7.5/tk virus genome.

81. (previously presented) The method of claim 71, wherein said isolated virus genome is a vEL/tk virus genome.

82. (previously presented) The method of claim 70, wherein the 5' and 3' flanking regions of said transfer plasmids are capable of homologous recombination with a vaccinia virus thymidine kinase gene.

83. (previously presented) The method of claim 82, wherein the 5' and 3' flanking regions of said transfer plasmids are capable of homologous recombination with a vaccinia virus HindIII J fragment.

84. (previously presented) The method of claim 82, wherein said transfer plasmids comprise heterologous nucleic acid molecules ligated into a plasmid selected from the group consisting of:

(a) p7.5/ATG0/tk which comprises SEQ ID NO:6,

(b) p7.5/ATG1/tk which comprises SEQ ID NO:7,

(c) p7.5/ATG2/tk which comprises SEQ ID NO:8, and

(d) p7.5/ATG3/tk, which comprises SEQ ID NO:9.

85. (previously presented) The method of claim 39, wherein said host cells are part of a monolayer, and wherein the floating host cells which are undergoing a lytic event are released from said monolayer.

86. (previously presented) The method of claim 39, wherein said MHC molecule is a class I MHC molecule.

87. (previously presented) The method of claim 46, wherein said host cells are part of a monolayer, and wherein the floating host cells which are undergoing a lytic event are released from said monolayer.

88. (previously presented) The method of claim 46, wherein said MHC molecule is a class I MHC molecule.

89. (previously presented) The method of claim 85, wherein (b) comprises recovering said host cells which are undergoing a lytic event as floating cells.

90. (previously presented) The method of claim 87, wherein (b) comprises recovering said host cells which are undergoing a lytic event as floating cells.

91. (previously presented) The method of claim 46, wherein (b) comprises recovering said host cells which are undergoing a lytic event as floating cells.

92. (previously presented) The method of claim 46, wherein (f) comprises recovering said host cells which are undergoing a lytic event as floating cells.

93-98. (cancelled)